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Environmental Influence on the Dominance of Phosphate-Solubilizing Bacteria (Bacillus *subtillis*) and Mycorrhizal Fungi (*Acaulospora* spp. and *Glomus spp.)* on the former mining land of PT. SIG TUBAN

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Abstract

Post-mining land generally experiences a decline in soil quality, which impacts microbial activity. This study aims to analyze the influence of environmental factors and soil physicochemical properties on the dominance of phosphate-solubilizing bacteria (*Bacillus subtilis*) and arbuscular mycorrhizal fungi (AMF) in the post-mining land of PT Semen Indonesia (SIG) Tuban. The study was conducted in three zones: Zone A (new excavation), Zone B (transition), and Zone C (revegetation). The parameters observed included environmental factors, soil physicochemical properties, Bacillus subtilis population, AMF spore count, and root colonization. Results showed that the highest Bacillus subtilis population was found in Zone B, at 5.2 × 10^6 CFU/g of soil. In contrast, the highest AMF spores (360 spores/100 g of soil) with 68% root colonization were found in Zone C. Soil conditions with a pH of 7.0, 3.5% organic carbon, and 50% porosity were shown to support the development of these microorganisms. Therefore, revegetation combined with the addition of organic materials and the use of AMF and Bacillus subtilis is recommended to accelerate soil fertility restoration in post-mining land.

Keywords: *Bacillus subtilis*, Mining Land, Mycorrhiza, Physicochemical Properties, Reclamation

1. Introduction

Open-pit mining by cement companies and other industries has caused extensive disturbances to the land environment, particularly the loss of topsoil layers, disrupted soil structure, reduced organic matter, and changes in temperature, humidity, and pH that are unfavorable for soil microorganisms (Zhakypbek et al., 2025).

These soils typically exhibit acidic pH, high surface temperatures, low organic matter content, and depressed microbial populations due to extreme conditions (Changey et al., 2022). These conditions bind nutrients like phosphorus (P) to aluminum (Al) and iron (Fe), making them less available to plants. Additionally, nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg) become less accessible. Furthermore, soil compaction resulting from heavy equipment activity and high aluminum content inhibits root growth, reducing the plant's ability to absorb water and nutrients. The low organic matter content also suppresses the activity of soil microorganisms involved in decomposing organic matter and recycling nutrients. As a result of these combined factors, plant growth is suboptimal, and agricultural productivity on ex-mining land tends to be low.

In Indonesia, a study on the identification of microorganisms in reclaimed land showed that the diversity and activity of soil bacteria are closely related to the soil's physicochemical conditions and the presence of revegetation vegetation (Nurtjahyani et al., 2018). These bacterial communities play a central role in soil ecosystem processes, such as organic matter decomposition, nutrient mineralization, nitrogen fixation, and the formation of soil aggregate structures. In the context of post-mining land, bacterial populations are often used as indicators of

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reclamation success because they are sensitive to environmental changes and can reflect the state of soil ecosystem recovery (Mao et al., 2024).

Several studies show that the long-term effectiveness of reclamation can be seen from the changes and benefits to the community. bacteria. Changes in the number of microbes reported by (Darma et al., 2022), that the addition of vermicompost fertilizer to peat soil, especially in the P2 treatment, was able to increase the total bacterial population to 6.17×10^6 cfu/ml, the total fungal population to 8.8×10^5 cfu/ml, Rastono & Muzadi's research, (2025), showed that *Bacillus subtilis* and AMF were able to increase dry weight by 23.253 grams, a net assimilation rate of 2.7950, and a plant growth rate of 9.790 in dry land compared to chili plants that were not treated. Finally, Hou et al. (2018) stated that the structure of the soil bacterial community on ex-coal mining land during 15 years of reclamation was able to restore the bacterial community to close to natural soil conditions so that it can be an indicator of improving soil quality. This is different from the research conducted, namely to determine the influence of the environment on microorganisms in post-mining land so that it can be a reference in land improvement efforts based on biological agents.

In post-mining areas in Indonesia, particularly in the region managed by PT. Semen Indonesia in Tuban Regency, three zones at different stages of recovery are identified: the newly mined zone, the transition zone, and the revegetated zone. The environmental conditions across these zones—such as air temperature, soil temperature, light intensity, soil pH, wind speed, and humidity—are believed to influence the growth and presence of soil bacteria. For example, research in Belitung indicated that differences in reclamation age significantly affect bacterial community composition and soil physical-chemical properties (Putra et al., 2024). Additionally, a national study found that nitrogen-fixing bacteria can be isolated from limestone reclamation soils, and that the environmental conditions of the rhizosphere greatly influence their presence (Fulyani & Purnomo, 2022).

Therefore, this study is crucial for quantitatively investigating how each environmental parameter in the land used for mining by PT. Semen Indonesia in Tuban Regency influences soil bacterial growth. The results are expected to provide a scientific basis for developing more effective reclamation and revegetation strategies and for enhancing understanding of soil microbial dynamics in post-mining areas.

2. Material and Methods

2.1. Location And Time Study

The research was conducted on former mining land owned of PT Semen Indonesia (Limited) Tbk., Temandang Village, Kerek District, Tuban Regency, East Java, with an altitude of 116 meters above sea level. The research location is divided in three zones based on the land restoration stage: Zone A (new excavation) -6.849577, 111.941038; Zone B (transition) -6.850515, 111.934343; Zone C (revegetation) -6.855031, 111.944257. The research was conducted from September 2025 to January 2026.

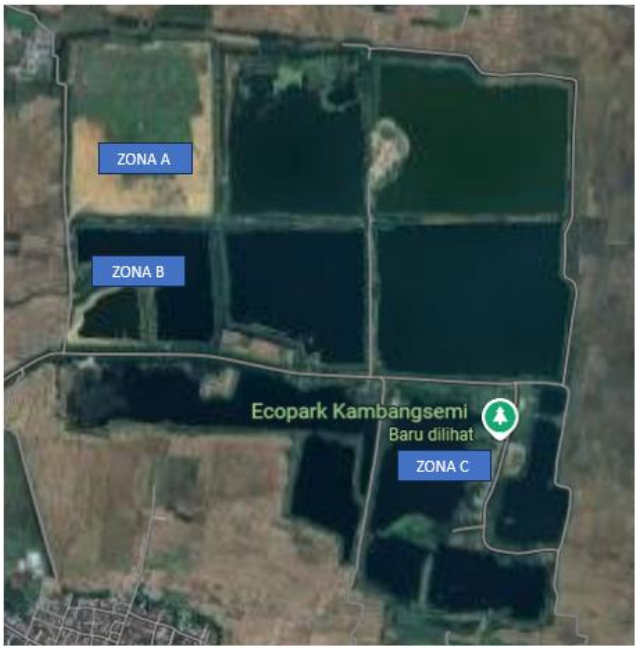


Figure 1. Research sample collection location

2.2. Tool And Material

The tools used included a thermometer (air and soil), lux meter, anemometer, hygrometer, digital pH meter, sterile soil drill, analytical balance, autoclave, incubator, and microbiology equipment (Petri dish, micropipette, Nutrient Agar (NA) culture media, 0.85% NaCl solution). Chemicals for sterilization, such as 70% alcohol, were also used.

2.3. Design Study

Samples were taken by purposive sampling from 3 land zones. Each zone had 3 sample points, resulting in 9 samples (replications). Soil samples were taken from the top layer (0-30 cm), which is the zone of microorganism activity, using a sterile soil auger; then the samples were mixed (composite) to represent each zone. Environmental observations at 3 location points, such as temperature, humidity, pH, wind speed, light intensity, and sampling point coordinates, were recorded 3 times per zone, resulting in 9 observations (Pratiwi & Nurcholis, 2023).

2.4. Identification

We carried out biological analysis or isolation of soil microorganisms to determine the total population *of Bacillus subtilis*, the number of Arbuscular Mycorrhizal Fungi (AMF) spores, and the percentage of AMF

colonization in each research zone. *The Bacillus subtilis population was calculated using a serial dilution* method up to 10^{-6}, followed by the *Total Plate Count* (TPC) method: 1 mL suspension was plated on Nutrient Agar (NA) medium, then incubated at 30°C for 48 hours, and the results were expressed as CFU/g soil (Erfandi, 2020). Meanwhile, fungal isolation was performed on PDA (Potato Dextrose Agar) medium using a 10^-6 serial dilution, and cultures were incubated for 7 days. The number of AMF spores was calculated using the wet sieving and decanting method and expressed as the number of spores per 100 g of soil, while the percentage of AMF colonization was determined by staining the roots using Trypan Blue and calculated using the GridlineIntersect method (Yefriwati et al. 2022).

2.5. Physicochemical Analysis

This analysis aims to understand soil characteristics in assessing the feasibility and need for soil rehabilitation. Observation parameters include pH analysis measured with a pH meter, Al-dd with a volumetric method, total N with the Kjeldahl method, available P with the Bray II method, exchangeable K with the leaching method with ammonium acetate pH 7, and organic C with the Walkley and Black method (Wisnubroto et al., 2024).

2.6. Data analysis

The data were analyzed using Analysis of Variance (ANOVA) and continued with the DMRT Test, followed by Pearson correlation to determine the relationship between the environment and soil physicochemical properties and the abundance of dominant bacteria (Pratiwi & Nurcholis, 2023).

2.7. Research Flow

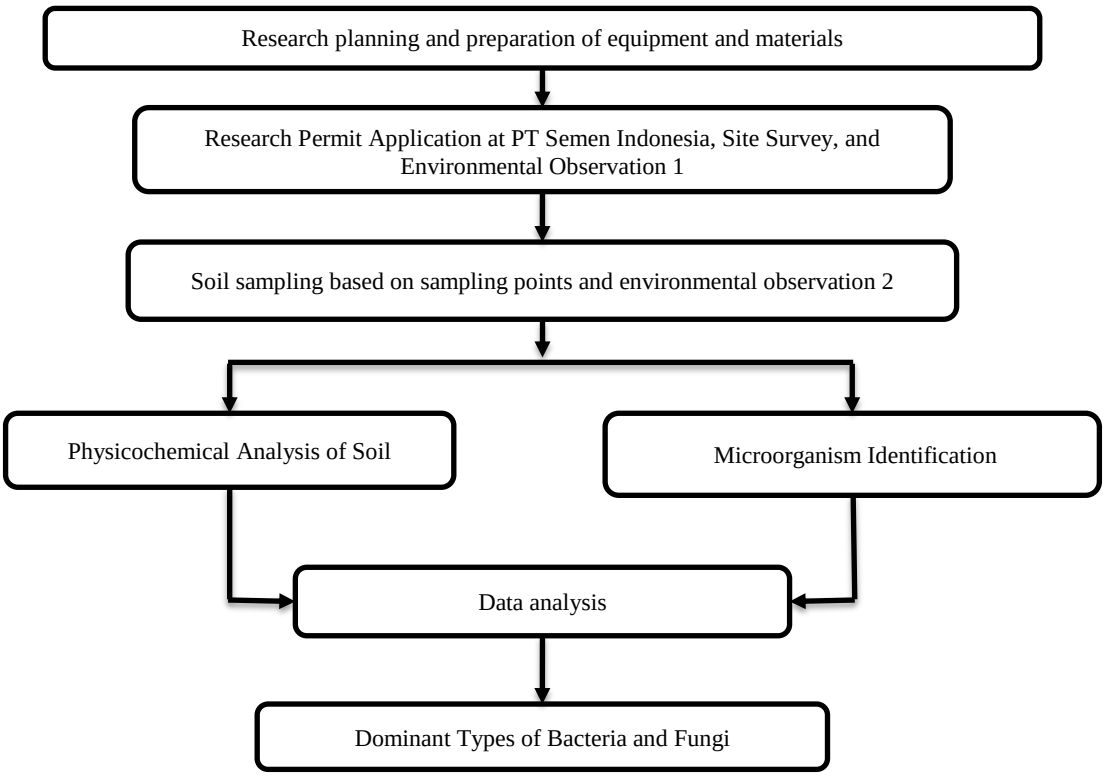


Figure 1. Research flow diagram

3. Results and Discussion

3.1. Measurement of environmental factors

Results: Environmental factors that can influence the number of microorganisms in ex-mining land at PT. Cement Indonesia Gresik (Kerek – Tuban) are outlined in Table 1.

Measurement of environmental factors in the three zones of PT. SIG Tuban's former mining land showed significant differences (Table 1). Zone A (New Quarry) had the highest air temperature of 37 ℃ and soil temperature of 33 ℃, while Zone C (Revegetation) had the lowest temperature (28 ℃ air and 25 ℃ soil). Light intensity in Zone A was also higher (1860 lux) than in Zone C (1650 lux). Wind speed decreased from 13 m/s in Zone A to 5 m/s in Zone C, while air humidity increased from 50% to 75%. Soil pH varied significantly across zones, ranging from pH 5.0 (Zone A) to pH 7.0 (Zone C).

These differences in environmental conditions have important implications for the existence of soil microorganisms. High temperatures and low humidity in Zone A can limit the growth of phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi (AMF).

Table 1. Analysis of environmental factors

Environmental Factor Parameters	Zone A (excavation New)	Zone B (Transition)	Zone C Results (Revegetation)
Temperature Air (℃)	37.00 ± 0.58 [a]	32.00 ± 0.58 [b]	28.00 ± 0.58 [c]
Temperature Land (℃)	33.00 ± 0.58 [a]	30.00 ± 0.58 [b]	25.00 ± 0.58 [c]
Light Sun (Lux)	1860.00 ± 1.15 [a]	1788.00 ± 1.15 [b]	1650.00 ± 1.15 [c]
pH	5.00 ± 0.06 [c]	5.90 ± 0.06 [b]	7.00 ± 0.06 [a]
Speed Wind	13.00 ± 0.58 [a]	9.00 ± 0.58 [b]	5.00 ± 0.58 [c]
Humidity	50.00 ± 0.58 [c]	60.00 ± 0.58 [b]	75.00 ± 0.58 [a]

Numbers in the same row followed by the same letter are not significantly different (P>0.05) according to the 5% LSD test.

Conversely, the more moderate conditions in Zone C, with near-neutral pH and high humidity, support fungal colonization and microbial activity that contribute in soil ecosystem recovery. These conditions in Zone C are consistent with research by Moses (2026), who stated that *Bacillus subtilis* can grow at temperatures around 25 °C with a pH of 7.00 and a humidity of 70%. Ziliwu and Lase (2025) also stated that soil microorganisms are very sensitive to changes in temperature, pH, and humidity.

3.2. Soil Physicochemical Measurements

This soil physicochemical analysis was conducted to understand the characteristics of the soil in assessing the feasibility and need for land rehabilitation. The physical and chemical properties of the soil were assessed to determine the condition of the soil in each zone of the ex-mining land. The parameters observed included bulk density, porosity, soil texture, soil color, pH, total N, available P, K-dd, and organic C as indicators of soil quality and fertility. These values were obtained by taking samples in 3 zones of the ex-mining land of PT. SIG, with each zone sampled at 3 points, resulting in 9 samples. Table 2 presents the analysis results..

Table 2. Soil Physico-Chemical Analysis

Parameter	Zone A	Zone B	Zone C
Bulk density (g/cm³)	1.45 ± 0.02[a]	1.35 ± 0.03[b]	1.20 ± 0.02[c]
Porosity (%)	42.0 ± 0.88[c]	45.0 ± 0.67[b]	50.0 ± 0.58[a]
Sand (%)	60.0 ± 0.58[a]	55.0 ± 0.88[b]	50.0 ± 1.20[c]
Silt (%)	25.0 ± 0.58[c]	30.0 ± 0.88[b]	35.0 ± 0.67[a]
soil pH	5.00 ± 0.09[c]	5.90 ± 0.07[b]	7.00 ± 0.05[a]
N-total (%)	0.08 ± 0.004[c]	0.12 ± 0.005[b]	0.18 ± 0.006[a]
P-available (mg/kg)	5.2 ± 0.31[c]	12.5 ± 0.46[b]	20.0 ± 0.38[a]
K-dd (mg/kg)	60 ± 1.53[c]	85 ± 1.20[b]	120 ± 1.76[a]
C-organic (%)	1.20 ± 0.05[c]	2.00 ± 0.08[b]	3.50 ± 0.10[a]

Numbers in the same row followed by the same letter are not significantly different (P>0.05) according to the 5% LSD test.

Physicochemical analysis of the soil showed an improvement in soil quality from Zone A to Zone C (Table 2). Soil density decreased from 1.45 g/cm³ in Zone A to 1.20 g/cm³ in Zone C, while porosity increased from 42% to 50%. Sand content decreased, while silt content increased, indicating a more balanced soil texture in the revegetation zone. In additi on, organic matter content increased from 1.2% in Zone A to 3.5% in Zone C.

Total N and available P contents followed a similar trend, indicating a gradual increase in soil fertility. Correlations between soil physicochemical variables and microbial counts (Table 4) showed a very strong relationship ($r \geq 0.97^{**}$) between AMF spores and soil pH, organic matter content, total N, available P, and porosity. Meanwhile, soil specific gravity and sand content were negatively correlated with AMF growth and phosphate-solubilizing bacteria.

The results of this study are similar to those of Zhang et al. (2016), which stated that revegetated land can increase soil organic carbon (SOC) content by up to 120.4% compared to land that experienced natural recovery, accompanied by a decrease in bulk density of 13.6% and an increase in soil aggregate stability. These results are consistent with the research of DeLong et al. (2012), which reported that natural forest soil has a bulk density of around 1.05 g cm^{-3}, while ex-mining soil reaches 1.70–1.84 g cm^{-3}. This indicates that more fertile, more porous soil, with a neutral pH and high organic content, supports the dominance of mycorrhizal fungi and soil bacteria (Zheng et al., 2019).

3.3. Bacterial and Fungal Analysis Results

The results of calculating the number of phosphate-solubilizing bacteria (Bacillus subtilis) and mycorrhizal fungi (AMF) showed different patterns in each zone (Table 3).

Phosphate-solubilizing bacteria (*Bacillus subtilis*): the highest number was found in Zone B (Transition) at 5.2×10^6 CFU/g, higher than Zone A (2.3×10^6 CFU/g) and Zone C (1.8×10^6 CFU/g). This indicates that phosphate-solubilizing

bacteria are more adapted to transitional environmental conditions, where temperature, pH, and humidity approach optimal levels. However, organic matter content has not yet reached its maximum. This finding is consistent with Yang et al. (2018) which states that soil bacteria can show a peak of dominance in transitional conditions when land begins to be reclaimed. Arbuscular mycorrhizal fungi (AMF) spores increased progressively from 85 spores/100 g in Zone A to 360 spores/100 g in Zone C, with the percentage of root colonization increasing from 18% to 68%. This result is in accordance with the research results (Wisnubroto et al., 2024), which stated that in former coal mining land, the number of AMF spores ranged from 140 spores per 100 g of soil, with the highest abundance of *Glomus* spp. found on flat slopes that have a relatively higher pH and cation exchange capacity (CEC), while on steep land caused by former excavations, it ranged from 105 per 100 g of soil, and the most frequently appearing was *Acaulospora* spp. This trend shows that mycorrhizal fungi are strongly influenced by stable soil conditions, rich in organic matter, and near-neutral pH, according to their role in helping nutrient absorption for revegetation plants (Nurtjahyani et al., 2018). The samples used in the study were 3 sample points per zone, so that there were 9 test samples.

3.4. Correlation of the number of microorganisms with physicochemical and environmental factors

Pearson correlation analysis (Table 4) shows clear interactions between environmental factors, soil characteristics, and microorganism populations.

Table 3. Results of analysis of the distribution of dominant bacteria and fungi in the former mining land of PT. Semen Indonesia Gresik (SIG) Tbk.

Zone	Total *Bacillus subtilis* (CFU/gr)	Dominant FMA Type	AMF Spore Count (spores/100 g soil)	Percentage of AMF Colonization (%)
Zone A	2.3×10^6	*Acaulospora* spp.	85	18 %
Zone B	5.2×10^6	*Glomus* spp.	245	46 %
Zone C	1.8×10^6	*Glomus* spp.	360	68 %

Table 4. Results of the correlation analysis of microorganisms with physicochemical and environmental factors

Variables	AMF spores	Colony	BD	Porosity	Sand (%)	Dust (%)	Soil pH	N-total	P-available	K-dd	C-Organic
AMF spores	1										
Colony	0.99**	1									
BD	-0.99**	-0.98**	1								
Porosity	0.99**	0.98**	-0.99**	1							
Sand (%)	-0.99**	-0.97**	0.99**	-0.99**	1						
Dust (%)	0.99**	0.98**	-0.98**	0.99**	-0.97**	1					
Soil pH	0.98**	0.97**	-0.97**	0.98**	-0.95*	0.96*	1				
N-total (%)	0.99**	0.98**	-0.98**	0.99**	-0.97**	0.98**	0.97**	1			
P-available (mg/kg)	0.99**	0.98**	-0.98**	0.98**	-0.97**	0.97**	0.97**	0.99**	1		
K-dd (mg/kg)	0.99**	0.97**	-0.97**	0.98**	-0.96*	0.97**	0.96*	0.98**	0.98**	1	
C-Organic (%)	0.99**	0.98**	-0.98**	0.99**	-0.97**	0.98**	0.98**	0.99**	0.99**	0.98**	1

*significant at the 5% significance level and **significant at the 1% significance level

AMF spores and the percentage of root colonization have a very high positive correlation (r = 0.99**), confirming that the more spores, the higher the ability of fungi to form symbiosis with roots. The number of phosphate-solubilizing bacteria is positively correlated with soil pH (r = 0.97**), total N (r = 0.98**), available P (r = 0.98**), and organic C (r = 0.98**), but decreases in soils with high specific gravity and high sand content. Extreme environments, such as high temperatures, intense light, and high wind speeds in Zone A, tend to inhibit the growth of AMF and some soil bacteria. At the same time, moderate conditions in Zones B and C favor the dominance of these microbes. These results are consistent with those described by (Ahmad et al., 2026), which showed that the three main components in the PCA analysis were able to explain 88% of the data variation with the largest contribution in the first component (61%) in line with the results of this study which showed a very strong correlation between environmental factors and soil physicochemical properties with the dominance of soil microorganisms, indicated by a high positive relationship between AMF spores and colonization (r = 0.99), as well as between the number of phosphate-solubilizing bacteria with soil pH, total N, available P, and organic C (r = 0.97–0.98). These findings support the research hypothesis that environmental factors and soil physicochemical quality influence the dominance of phosphate-solubilizing bacteria and mycorrhizal fungi in the former mining land of PT. SIG Tuban. After describing

the results of soil physical and chemical parameter measurements in Table 4, we carried out a Pearson correlation analysis to determine the relationship between environmental variables and the abundance of arbuscular mycorrhizal fungi (AMF) spores. The in below shows the correlation results in a heatmap (Figure 3).

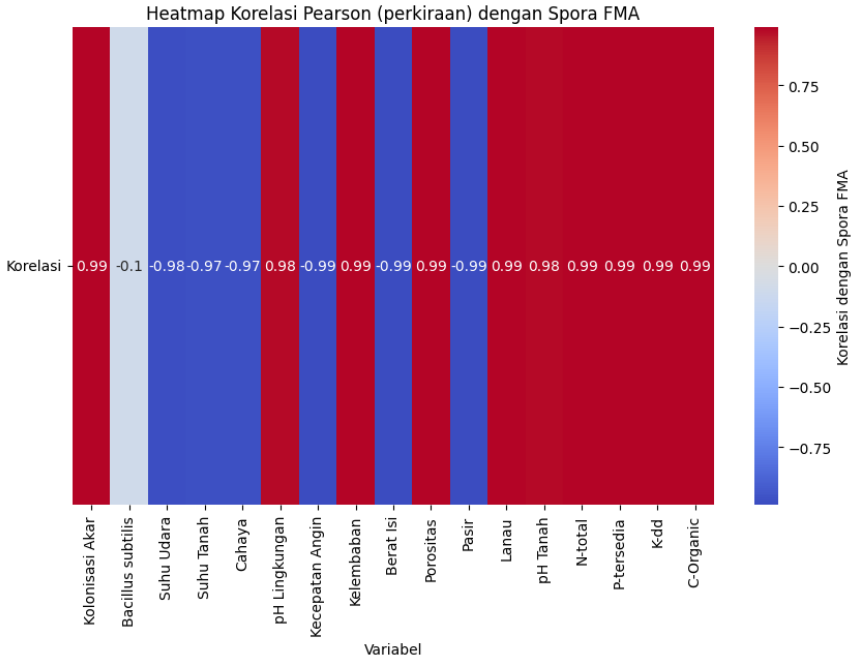


Figure 3. Heat map of Pearson Correlation Results

From the heatmap, it can be seen that most of the soil physical and chemical variables, such as root colonization, the number of *Bacillus subtilis*, soil pH, moisture, bulk density, porosity, sand content, and nutrient levels (total N, available phosphorus, exchangeable potassium, and organic carbon), have a very strong positive correlation with the abundance of AMF spores, with a correlation value approaching +0.99. This indicates that improving the quality of soil physicochemical properties directly contributes to increasing the population of arbuscular mycorrhizal fungi in the soil.

Soil conditions with sufficient nutrient availability, high organic matter content, and good soil structure are known to increase root colonization and AMF spore density in the plant rhizosphere. Furthermore, the presence of AMF also plays a crucial role in increasing soil fertility and supporting revegetation in post-mining areas through symbiosis with plants (Asmarahman et al., 2018).

Kumar et al.'s (2018) research showed that during the coal mine reclamation process for 1–26 years, soil organic carbon content increased by 6.6-fold and easily extractable glomalin increased by 11.5-fold, which was followed by an increase in AMF spore density and was strongly correlated with organic carbon, pH, total nitrogen, and microbial biomass. These results are supported by Yang et al. (2018), who reported that the addition of compost can increase AMF spore density by 45–60% and increase root colonization by increasing organic matter and soil nutrient availability.

In contrast, the variables of soil temperature, light intensity, wind speed, and silt content showed a very strong negative correlation with AMF spores, with correlation values around −0.97 to −0.99. Zhang et al. (2016) reported that increasing temperature caused AMF spore density to decrease by 32% and spore diameter to decrease by 10%. Similarly, Panwar et al. (2011) showed that optimal AMF root colonization occurred at soil temperatures of 15.4–24.4°C, with the highest spore density in the range of 20.8–28.8°C.

This result suggests that extreme environmental conditions can inhibit the development of mycorrhizal fungi in the soil. Excessively hot and open environments in post-mining areas can reduce soil microbial activity and inhibit spore formation and root colonization by AMF (Tomo & Prasetya, 2021). Meanwhile, air temperature showed a very weak correlation (−0.1), indicating it had no significant effect on AMF spore abundance at the study site.

4. Conclusion

Revegetation has been shown to improve soil quality in post-mining areas by enhancing pH, porosity, organic matter content, and nutrient levels. These improvements increase arbuscular mycorrhizal fungi (AMF) abundance to 360 spores per 100 g of soil and root colonization rates of 68%. Key factors supporting the development of soil microorganisms include pH, organic carbon, total nitrogen, available phosphorus, and porosity. Therefore, the combined application of revegetation, organic matter

management, and the use of biological agents such as AMF and Bacillus subtilis is recommended to accelerate soil fertility recovery in post-mining areas.

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